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(54) hTFIIIA gene

(57) The present invention provides a hTFIIIA gene containing a base sequence coding for the amino acid sequence shown under SEQ ID NO:1, in particular a hTFIIIA gene containing the base sequence shown under SEQ ID NO:2.

The gene can express a corresponding hTFIIIA protein. The gene and protein serve as transcription regulating factors and are useful in the diagnosis or identification of hereditary diseases such as cancer or other diseases resulting from abnormal transcriptional control and, further, in analyzing the mechanisms of action thereof.

EP 0 704 526 A1

Description

TECHNICAL FIELD

The present invention relates to a gene coding for human transcription factor IIIA (hereinafter referred to as hTFIIIA).

BACKGROUND ART

Since TFIIIA was purified as a transcription factor for the first time in 1980 from Xenopus oocytes [Segall et al., J. Biol. Chem., 255, 11986-11991 (1980)], a number of in vivo and in vitro studies have been made in Xenopus for elucidating the mechanism of transcriptional control by said TFIIIA [e.g. Del et al., Nucleic Acids Res., 19, 6197-6203 (1991); Smith et al., Nucleic Acids Res., 19, 6871-6876 (1991); Liao et al., J. Mol. Biol., 223, 857-871 (1992); Del et al., J. Mol. Biol., 233, 567-579 (1993)].

The above-mentioned Xenopus TFIIIA is necessary for the initiation of 5S RNA gene transcription [Sakonji et al., Cell 19, 13-25 (1980)] and binds to an internal control region of the 5S gene [Bogenhagen et al., Cell, 19, 27-35 (1980)].

The nucleic acid sequence of the Xenopus TFIIIA cDNA and the corresponding amino acid sequence have already been reported [Ginsberg et al., Cell 39, 479-489 (1984)]. Said gene codes for nine zinc finger domains (repetitions of the Cys_2His_2 (C_2H_2) motif), and this structure is regarded as an essential domain for a group of DNA-binding proteins [Miller et al., EMBO J., 4, 1607-1614 (1985)].

It has been established that a yeast gene coding for a protein homologous to the Xenopus TFIIIA also has the same C_2H_2 motif [Archambault et al., J. Biol. Chem., 267, 3283-3288 (1992)].

It is further known that, in human, DNA binding transcriptional factors such as the human Wilms tumor gene WT1 [Gessler et al., Nature, 343, 774-778 (1990)], the human transcriptional repressor YY1 [Shi et al., Cell, 67, 377-388 (1991)], the human MYC-associated zinc finger protein maz [Bossone et al., Proc. Natl. Acad. Sci., USA, 89, 7452-7456 (1992)] and sp1 [Kuwahara et al., Biolchem., 29, 8627-8631 (1990)] have finger domains of the above C_2H_2 type.

In contrast to Xenopus TFIIIA, little is known about hTFIIIA. Thus, while, in 1989, a hTFIIIA-like protein (35kDa protein) was purified from HeLa cells and its inter-action with the human 5S RNA gene was shown [Seifart et al., J. Biol. Chem., 264, 1702-1709 (1989)], no hTFIIIA-encoding gene has been reported as yet.

Accordingly, it is an object of the present invention to isolate and provide a hTFIIIA gene.

Another object of the present invention is to reveal the nucleic acid sequence of the hTFIIIA gene and the corresponding amino acid sequence and thereby shed light on the human transcriptional mechanism and provide a use thereof.

DISCLOSURE OF THE INVENTION

As a result of their intensive investigations, the present inventors successfully isolated a cDNA coding for hTFIIIA, determined the whole cDNA sequence and the corresponding amino acid sequence, caused its expression in various tissues and revealed its locus on chromosome. Based on the findings thus obtained, the present invention has now been completed.

Thus, the present invention provides a hTFIIIA coding for an amino acid sequence defined by SEQ ID NO:1.

Hereinafter, in the present specification, abbreviations are used for amino acids, peptides, base sequences, nucleic acids and so forth as recommended by IUPAC and IUB and in "Guideline for drafting specifications etc. which contain base sequences and/or amino acid sequences" (edited by Japanese Patent Office) or conventionally used in the relevant field of art.

The hTFIIIA gene of the present invention has an open reading frame comprising 1269 nucleotides (nucleic acids) coding for 423 amino acid residues as shown under SEQ ID NO:1, and is characterized by coding for nine C_2H_2 type zinc finger domains. When compared with the Xenopus TFIIIA gene, it shows 63% homology with respect to nucleic acids and 58% homology with respect to amino acids.

The hTFIIIA encoded by the gene of the present invention supposedly plays a biological role as a DNA binding protein, and said gene is useful as a transcription regulating factor. In particular, the gene of the present invention is expressed generally in various tissues, hence presumably plays an important role in the initiation of 5S ribosomal RNA gene transcription and in the maintenance of the stability of transcription of other genes, thus being involved in life-supporting and controlling functions.

In particular, a very large number of diseases accompanying a disorder in transcriptional control have recently become known. For example, many of oncogene products act as transcription regulating factors, and disorders therein lead to canceration of cells. In promyelocytic leukemia, chromosomal translocation results in a disorder in transcriptional control, which in turn causes canceration. High-level expression of the regulatory factor Hox2.4 induces leukemia in mice. Thus, a number of hereditary diseases are now known in which a protein concerned shows no abnormality but the pathologic mechanisms of which involve an abnormality of a gene involved in the transcriptional control required for

the expression of the gene for said protein. By investigating these gene abnormalities (DNA diagnosis etc.), it is possible to identify hereditary diseases the pathogenetic analysis of which has not sufficiently advanced. The gene of the present invention is useful in such field. The gene of the present invention is also useful in the treatment of diseases through transcriptional control using an antisense or in analyzing the mechanisms of action thereof.

Furthermore, TFIIIA is involved in the transcriptional control of 5S RNA and, therefore, a disorder in this transcriptional control directly leads to a disorder in the synthesis of the protein concerned. Many hereditary diseases showing an abnormality in the quantity of a protein are presumably caused by such disorder in protein synthesis. Thus, the gene of the present invention is expected to be useful also in throwing light on such diseases.

While the gene of the present invention is represented in terms of a single-stranded DNA sequence, as shown under SEQ ID NO:2, the present invention includes, within the scope thereof, a DNA sequence complementary to such single-stranded DNA sequence and a component comprising both of them as tell. The DNA sequence shown under SEQ ID NO:2 and representing the gene of the present invention is an example of the combination of codons coding for respective amino acid residues according to the amino acid sequence shown under SEQ ID NO:1. The gene of the present invention is not limited thereto but, of course, can have any DNA base sequence that comprises some other arbitrary combination of codons for respective amino acid residues without altering the above amino acid sequence. The codon selection can be made in a conventional manner, for example taking into consideration the codon employment frequencies in the host to be used [Nucl. Acids Res., 9, 43-74 (1981)].

The gene of the present invention further includes DNA sequences coding for equivalents to the amino acid sequence mentioned above as modified therefrom by deletion and/or substitution of at least one amino acid or partial amino acid sequence thereof or by addition of at least one amino acid or amino acid sequence and having a biological activity similar to that of hTFIIIA. These equivalents may be produced spontaneously or can be produced by posttranslational modification or further can be produced (by modification, mutation, etc.) by modifying the natural gene (gene of the present invention) using such techniques as site-specific mutagenesis [Kramer, W., et al., Nucl. Acids Res., 12, 9441 (1984); Kramer, W. and Frits, H. J., Methods in Enzymology, 154, 350 (1987); Zoller, M. J. and Smith, M., Methods in Enzymology, 100, 468 (1983); Hirose, Susumu, Seikagaku Jikken Koza (Experiments in Biochemistry), 2nd series, vol. 1, "Idenshi Kenkyu-ho (Methods in Genetic Studies) II". 105], by synthesizing modified DNAs using such chemical synthesis techniques as the phosphotriester method [Letsinger, R. L. and Ogilvie, K. K., J. Am. Chem. Soc., 91, 3350 (1969); Merrifield, R. B., Science, 150, 178 (1968)] and the phosphoamidite method [Beaucage, S. L. and Caruthers, M. H., Tetrahedron Lett., 22, 1859 (1981); McBride, L. J. and Caruthers, M. H., Tetrahedron Lett., 24, 245 (1983)], or by a combination of these.

By utilizing the gene of the present invention, namely inserting it, for example, into a microbial vector and cultivating the thus-transformed microorganism, it is possible to cause expression of hTFIIIA with ease and in large quantities and thereby isolate and provide said protein.

The gene of the present invention can be readily produced based on the sequence information on the gene of the present invention as disclosed herein, using general genetic engineering techniques [e.g. Sambrook, J., Fritsch, E. F., Maniatis, T., Molecular Cloning, 2nd edition, Cold Spring Hrbor Laboratory Press (1989); Seikagaku Jikken Koza, 2nd series, "Idenshi Kenkyu-ho I, II, III", edited by Nippon Seikagaku-Kai; Guide to Molecular Cloning Techniques, Berger, S. L, Kimmel, A. R., Methods in Enzymology, vol. 152], among others.

For example, said gene can be produced by selecting, from among a human cDNA library (prepared in a conventional manner from appropriate origin cells containing a gene coding for hTFIIIA), a desired clone using an appropriate probe or antibody specific to the gene of the present invention [cf. e.g. Sugga, S. V., et al., Proc. Natl. Acad. Sci., USA, 78, 6613 (1981); Young, R. A., et al., Science, 222, 778 (1983)].

As examples of the origin cells to be used in the above procedure, there may be mentioned various cells and tissues, and cultured cells derived therefrom, which allow expression of the hTFIIIA gene. Whole RNA separation from these, mRNA separation and purification, and conversion to (synthesis of) cDNA and cloning of the same and other steps can be performed in the conventional manner. Furthermore, cDNA libraries are commercially available and, in the practice of the present invention, such cDNA libraries, for example various cDNA libraries available from Clontech, can also be used.

Screening of the gene of the present invention from such a cDNA library can be carried out in the conventional manner, as mentioned above. As the method of screening, there may be mentioned, for example, the method comprising the use of an anti-hTFIIIA specific antibody against the protein produced by the cDNA and thus selecting a corresponding cDNA clone by means of Western blotting, the method comprising Southern blotting using a probe selectively binding to the objective DNA sequence, the Northern blotting method, and a combination of these. Generally, a DNA sequence chemically synthesized based on the information on the DNA sequence of the gene of the present invention, for instance, is used here as the probe. Of course, it is also possible to use the gene of the present invention already obtained or a fragment thereof as such a probe.

In obtaining the gene of the present invention, the DNA/RNA amplification method comprising the PCR technique [Saiki, R. K., et al., Science, 230, 1350-1354 (1985)] can also be used successfully. Particularly in cases where a full-length cDNA cannot be obtained from the library, the technique of RACE [Rapid Amplification of cDNA Ends; Jikken

Igaku, 12 (6), 35-38 (1994)] can suitably be employed. The primers to be used in employing such PCR technique can appropriately be designed based on the sequence information on the gene of the present invention and can be synthesized by a per se known conventional method.

The amplified DNA/RNA fragment can be isolated and purified in the conventional manner, as mentioned above, for example by gel electrophoresis.

The base sequence of the gene of the present invention or of any of various DNA fragments thereof can be determined in the conventional manner, for example by the dideoxy method [Sanger, F., et al., Proc. Natl. Acad. Sci., USA, 74, 5463-5467 (1977)] or the Maxam-Gilbert method [Maxam, A. M. et al., Methods in Enzymology, 65, 499 (1980)]. Such base sequence determination can also be made with ease using a commercially available sequencing kit or the like.

The whole DNA base sequence of a cDNA thus obtained and named clone OTK7 and serving as an example of the gene of the present invention is as shown under SEQ ID NO:3, and the amino acid sequence of hTFIIIA encoded by said cDNA is as shown under SEQ ID NO:1.

In accordance with the present invention, a method of screening the hTFIIIA gene is provided which comprises using a part of the gene of the present invention as a probe. Here, the probe can be labeled, for example by using a random prime DNA labeling kit (available from Takara Shuzo, Amersham, etc.) which makes use of the random prime DNA labeling technique [Feinberg, A. P., et al., Anal. Biochem., 137 266-267 (1984)], and the objective gene can be screened, for example by the plaque hybridization technique [Benton, W., et al., Science, 196, 383-394 (1977)].

Furthermore, it is possible, starting with the gene of the present invention, to obtain recombinant hTFIIIA species in accordance with general gene recombination techniques [cf. e.g. Science, 224, 1431 (1984); Biochem. Biophys. Res. Comm., 130, 692 (1985); Proc. Natl. Acad. Sci., USA, 80, 5990 (1983)]. More specifically, said hTFIIIA species can be produced by constructing a recombinant DNA which allows expression of the gene of the present invention in host cells, introducing the same into the host cells for transformation and cultivating the thus-obtained transformant.

The host cells to be used may be either eukaryotic or prokaryotic. As the expression vector for vertebrate cells, use may be made of those which possess a promoter generally located upstream of the gene to be expressed, an RNA splicing site, a polyadenylation site and a transcription termination sequence and which may have a replication origin as necessary. As eukaryotic microorganisms, frequent use is generally made of yeasts and, among them, yeasts of the genus Saccharomyces can be used with advantage. As the expression vector for eukaryotic micro-organisms such as yeasts, use may be made of pAM82 having a promoter for the acid phosphatase gene [A. Miyanohara et al., Proc. Natl. Acad. Sci., USA, 80, 1-5 (1983)], for instance. As eukaryotic hosts, general and frequent use is made of Escherichia coli and Bacillus subtilis. When these are used as hosts in the practice of the present invention, it is desirable to use an expression plasmid constructed by inserting the gene of the present invention into a plasmid vector capable of replicating in said hosts in a manner such that said expression plasmid is provided, upstream of the gene of the present invention, with a promoter and the SD (Shine and Dalgarno) base sequence and further with an initiation codon (e.g. ATG) required for the initiation of protein synthesis so that said gene can be expressed. Escherichia coli K12, for instance, is frequently used as the host Escherichia coli mentioned above, with frequent use being generally made of pBR322 as the vector. These are, however, not limitative but other various per se known strains and vectors may also be used. Usable as the promoter are, for example, the tryptophan (trp) promoter, lpp promoter, lac promoter, P_L promoter, and the like.

The thus-obtained desired recombinant DNA can be introduced into host cells for transformation thereof by various methods generally employed in the art. The transformant obtained can be cultivated by a conventional method. The cultivation results in production and accumulation of the objective hTFIIIA encoded by the gene of the present invention. The medium to be used in said cultivation can be appropriately selected from among various media in common use according to the host cells employed, and the cultivation can be carried out under conditions suited for the growth of the host cells.

In the above manner, the objective recombinant hTFIIIA protein is produced and accumulated or secreted intracellularly or extracellularly of the transformant cells.

The recombinant hTFIIIA can be isolated and purified by various separation procedures utilizing its physical and/or chemical and/or other properties [cf. "Seikagaku (Biochemistry) Data Book", pages 1175-1259, 1st edition, 1st printing, published June 23, 1980 by Tokyo Kagaku Dozin; Biochemistry, vol. 25, No. 25, 8274-8277 (1986); Eur. J. Biochem., 163, 313-321 (1987)]. Concretely, said procedures include such conventional ones as reconstitution treatment, treatment with a protein precipitant (salting out), centrifugation, osmotic shock procedure, ultrasonication, ultrafiltration, molecular sieve chromatography (gel filtration), adsorption chromatography, ion exchange chromatography, affinity chromatography, high-performance liquid chromatography (HPLC), other chromatographic techniques, dialysis, and combinations of these, among others. In the above manner, the desired recombinant hTFIIIA can be produced on a commercial scale with ease and in high yields.

In accordance with the present invention, a hTFIIIA gene is provided, and hTFIIIA can be produced with ease and in large quantities using said gene. The gene and hTFIIIA of the present invention are useful astranscription regulating factors and are useful, among others, in the diagnosis and identification of cancer and other hereditary diseases resulting from disorders in transcriptional control, in the treatment of such diseases by the transcriptional control, and in analyzing the mechanisms of action of such control.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows the results of Northern blotting for visualizing the expression of the gene of the present invention in various tissues.

EXAMPLES

The following examples are further illustrative of the present invention.

Example 1

(1) Cloning and sequencing

As a result of sequence analysis of clones arbitrarily selected from a human fetal brain cDNA library, a 1.3 kb clone showing a high level of homology to Xenopus TFIIIA was found and named OTK7-1. Sequence analysis revealed that this clone lacks a 5' portion of the gene.

(2) 5' RACE

A cDNA clone containing the 5' portion of the gene was isolated by 5' RACE using a commercial kit (5'-Ampli-FINDER™ RACE kit, Clontech).

In that case, three primers corresponding to OTK7-1, namely H11-R (the base sequence shown under SEQ ID NO:4), H11-E (the sequence shown under SEQ ID NO:5) and H11-H (shown under SEQ ID NO:6), and one primer (AP-2; shown under SEQ ID NO:7) complementary to an anchor primer (shown under SEQ ID NO:8) were synthesized.

A 300 ng portion of human brain poly A$^+$ RNA (Clontech) was reverse-transcribed with the primer H-11R for single-stranded cDNA synthesis.

Thus, 9 µl of poly A$^+$ RNA (300 ng/9 µl) and 1 µl of primer H11-R (10 picomoles/µl) were preincubated at 65°C for 5 minutes, a reaction mixture [9.2 µl of DEPC-treated H_2O/9 µl of 4 x reverse transcriptase buffer/1.6 µl of RNase inhibitor (40 units/µl)/3.7 µl of dNTPmix (10 mM each nucleotide)/0.5 µl of AMV reverse transcriptase (25 units/µl)] was added, and incubation was performed at 52°C for 30 minutes. The reaction was terminated by adding 10 µl of 0.5 M EDTA, the template poly A$^+$ RNA was then hydrolized by adding 10 µl of 6 N NaOH, and the excess primer H11-R was removed using a GENO-BIND™ system. Following precipitation with ethanol, the cDNA pellet was resuspended in 6 µl of H_2O.

Then, the single-stranded anchor oligonucleotide (anchor primer) was ligated to the 3' end of the above-mentioned cDNA using T4 DNA ligase, as follows.

A mixture composed of 2.5 µl of the above cDNA, 2 µl of the anchor primer (4 picomoles), 5 µl of 2 x ligation buffer and 0.5 µl of T4 DNA ligase (20 units/µl) was incubated at room temperature for 18 hours.

The ligated mixture was 10-fold diluted and used as a template for PCR.

A 1.0-µl portion of the anchor-ligated cDNA dilution was subjected to PCR for amplification using the primers AP-2 and H11-E, as follows.

Said portion was kept at 82°C for 1 minute, the primers were then added, and 35 PCR cycles were conducted (each cycle comprising keeping at 92°C for 0.5 minute, at 56°C for 0.5 minute and at 72°C for 1.0 minute), followed by 15 minutes of incubation at 72°C. The PCR products were cloned into the pBluescript SK(-) vector at the EcoRV site thereof. The desired transformants were selected by colony hybridization using ^{32}P-ATP end-labeled oligo H11-I. The positive colonies were subjected to sequence determination by the dideoxy termination method [Sanger et al., Proc. Natl. Acad. Sci., USA, 74, 5463-5467 (1977)].

The thus-obtained cDNA, which is a gene of the present invention is hereinafter referred to as "OTK7".

(3) Northern hybridization

The expression of the gene OTK7 of the present invention was examined in various tissues using a human multiple tissue Northern blot system (Clontech).

Thus, blots were subjected to 4 hours of pre-hybridization at 50°C in a solution comprising 50% formamide, 10 x Denhardt's solution, 5 x SSPE, 2% SDS and 100 µg/ml of denatured salmon sperm DNA, with [^{32}P]-labeled cDNA as a probe, followed by 18 hours of hybridization. The blots were washed, at room temperature, three times with 2 x SSC/0.05% SDS over 10 minutes and then two times with 0.1 x SSC/0.1% SDS over 15 minutes, and subjected to autoradiography at -80°C for 16 hours.

(4) Chromosome mapping

Chromosome mapping was performed in the manner of direct R-banding fluorescence in situ hybridization [FISH; Takahashi et al., Hum. Genet., 86, 14-16 (1990) and ibid., 88, 119-121 (1991)].

(5) Results

a) DNA sequence of OTK7 gene and corresponding amino acid sequence

The nucleotide sequence of the OTK7 cDNA and the corresponding amino acid sequence are shown under SEQ ID NO:3.

Referring to SEQ ID NO:3, the sequence consisting of the 1289th to 1291st bases is the termination codon (TAA), the sequence comprising the 317th to 1096th bases corresponds to the zinc finger domains, the sequence from the 20th to 22nd bases (ATG) is the initiation methionine codon, and the 1363rd to 1368th bases (ATTAAA) constitute a polyadenylation signal.

The OTK7 cDNA comprises a total of 1399 bases, inclusive of a 1269-base open reading frame coding for 423 amino acid residues.

As far as the 5' three fourths of its coding region is concerned, said cDNA showed 63% homology in nucleotides and 58% homology in amino acids to Xenopus TFIIIA.

Such hTFIIIA has nine zinc finger domains and the amino acid sequences thereof well conserve the C_2H_2 finger domains of Xenopus TFIIIA except for the 6th finger domain which has only 3 amino acid residues between two cysteine residues instead of 5 amino acid residues in the case of Xenopus TFIIIA.

In the C terminal region, the homology between the two is not so high. They differ also in the size of N terminal region. Whereas, in Xenopus TFIIIA, there are 14 amino acid residues upstream of the first finger domain, there are 99 amino acid residues in hTFIIIA. This N terminal region of hTFIIIA shows no homology to any of the so-far known gene products.

The homology of hTFIIIA to other known DNA binding proteins is limited to a relatively small region, as follows:

Xenopus 5S RNA binding protein p43 [Joho et al., Cell, 61, 293-300 (1990)] ··· out of 289 amino acid residues, 37% are identical;

Human Wilms tumor gene product WT1 [Gessler et al., Nature, 343, 774-778 (1990)] ··· out of 126 amino acid residues, 35% are identical;

Human transcriptional repressor YYA [Shi et al., Cell, 67, 377-388 (1991)] ··· out of 95 amino acid residues, 40% are identical;

Human GT box binding protein [Kingsley et al., Mol. Cell. Biol., 12, 4251-4261 (1992)] ··· out of 91 amino acid residues, 44% are identical;

Human myc-associated zinc finger protein [Bossone et al., Proc., Natl. Acad. Sci., USA, 89, 7452-7456 (1992)] ··· out of 152 amino acid residues, 37% are identical.

b) Northern blot analysis

The levels of expression of hTFIIIA in various tissues are shown in Fig. 1.

In Fig. 1, the results of the above-mentioned test (hTFIIIA expression) with a 1.1 kbp cDNA as a probe are shown in the upper row, and the results (controls) of a β-actin m-RNA detection test conducted for the same blots in the same manner using a β-actin probe are shown in the lower row. The lanes are respectively for the following:

Lane 1:	heart
Lane 2:	brain
Lane 3:	placenta
Lane 4:	lung
Lane 5:	liver
Lane 6:	skeletal muscle
Lane 7:	kidney
Lane 8:	pancreas
Lane 9:	spleen
Lane 10:	thymus
Lane 11:	prostate
Lane 12:	testis
Lane 13:	ovary
Lane 14:	small intestine

Lane 15: colon
Lane 16: peripheral blood leukocyte

The size of the hTFIIIA transcript was estimated at about 1400 bp upon Northern analysis. This size is almost in agreement with that of the OTK7 cDNA and, therefore, said cDNA presumably covers approximately the whole sequence of the hTFIIIA mRNA.

While this gene is ubiquitously expressed in all the human tissues tested, the level of expression seems higher in such tissues as pancreas, spleen and peripheral blood leukocyte than in other tissues.

c) Mapping

The hTFIIIA gene was found to reside on the chromosome 13q12.3-13.1.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Otsuka Pharmaceutical Co., Ltd.
(B) STREET: 9, Kandatsukasacho 2-chome, Chiyoda-ku
(C) CITY: Tokyo
(E) COUNTRY: Japan
(F) POSTAL CODE (ZIP): 101

(ii) TITLE OF INVENTION: hTFIIIA Gene

(iii) NUMBER OF SEQUENCES: 8

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: JP 211022/1994
(B) FILING DATE: 05-SEP-1994

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 423 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Met Arg Ser Ser Gly Ala Asp Ala Gly Arg Cys Leu Val Thr Ala Arg
1               5                  10                  15

Ala Pro Gly Ser Val Pro Ala Ser Arg Glu Gly Ser Ala Gly Ser Arg
            20                  25                  30

Gly Pro Gly Ala Arg Phe Pro Ala Arg Val Ser Ala Arg Gly Ser Ala
        35                  40                  45

Pro Gly Pro Gly Leu Gly Gly Ala Gly Ala Leu Asp Pro Pro Ala Val
    50                  55                  60

Val Ala Glu Ser Val Ser Ser Leu Thr Ile Ala Asp Ala Phe Ile Ala
65                  70                  75                  80

Ala Gly Glu Ser Ser Ala Pro Thr Pro Pro Arg Pro Ala Leu Pro Arg
                85                  90                  95
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Arg Phe Ile Cys Ser Phe Pro Asp Cys Ser Ala Asn Tyr Ser Lys Ala
            100                 105                 110

Trp Lys Leu Asp Ala His Leu Cys Lys His Thr Gly Glu Arg Pro Phe
        115                 120                 125

Val Cys Asp Tyr Glu Gly Cys Gly Lys Ala Phe Ile Arg Asp Tyr His
    130                 135                 140

Leu Ser Arg His Ile Leu Thr His Thr Gly Glu Lys Pro Phe Val Cys
145                 150                 155                 160

Ala Ala Asn Gly Cys Asp Gln Lys Phe Asn Thr Lys Ser Asn Leu Lys
                165                 170                 175

Lys His Phe Glu Arg Lys His Glu Asn Gln Gln Lys Gln Tyr Ile Cys
            180                 185                 190

Ser Phe Glu Asp Cys Lys Lys Thr Phe Lys Lys His Gln Gln Met Lys
        195                 200                 205

Ile His Gln Cys Gln Asn Thr Asn Glu Pro Leu Phe Lys Cys Thr Gln
    210                 215                 220

Glu Gly Cys Gly Lys His Phe Ala Ser Pro Ser Lys Leu Lys Arg His
225                 230                 235                 240

Ala Lys Ala His Glu Gly Tyr Val Cys Gln Lys Gly Cys Ser Phe Val
                245                 250                 255

Ala Lys Thr Trp Thr Glu Leu Leu Lys His Val Arg Glu Thr His Lys
            260                 265                 270

Glu Glu Ile Leu Cys Glu Val Cys Arg Lys Thr Phe Lys Arg Lys Asp
        275                 280                 285

Tyr Leu Lys Gln His Met Lys Thr His Ala Pro Glu Arg Asp Val Cys
    290                 295                 300

Arg Cys Pro Arg Glu Gly Cys Gly Arg Thr Tyr Thr Thr Val Phe Asn
305                 310                 315                 320

Leu Gln Ser His Ile Leu Ser Phe His Glu Glu Ser Arg Pro Phe Val
                325                 330                 335

Cys Glu His Ala Gly Cys Gly Lys Thr Phe Ala Met Lys Gln Ser Leu
            340                 345                 350

Thr Arg His Ala Val Val His Asp Pro Asp Lys Lys Lys Met Lys Leu
        355                 360                 365

Lys Val Lys Lys Ser Arg Glu Lys Arg Glu Phe Gly Leu Ser Ser Gln
    370                 375                 380

Trp Ile Tyr Pro Pro Lys Arg Lys Gln Gly Gln Gly Leu Ser Leu Cys
385                 390                 395                 400

Gln Asn Gly Glu Ser Pro Asn Cys Val Glu Asp Lys Met Leu Ser Thr
```

405 410 415

Val Ala Val Leu Thr Leu Gly
420

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1269 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATGCGCAGCA GCGGCGCCGA CGCGGGGCGG TGCCTGGTGA CCGCGCGCGC TCCCGGAAGT 60

GTGCCGGCGT CGCGCGAAGG TTCAGCAGGG AGCCGTGGGC CGGGCGCGCG GTTCCCGGCA 120

CGTGTCTCGG CACGTGGCAG CGCGCCTGGC CCTGGGCTTG GAGGCGCCGG CGCCCTGGAT 180

CCGCCGGCCG TGGTCGCCGA GTCGGTGTCG TCCTTGACCA TCGCCGACGC GTTCATTGCA 240

GCCGGCGAGA GCTCAGCTCC GACCCCGCCG CGCCCCGCGC TTCCCAGGAG GTTCATCTGC 300

TCCTTCCCTG ACTGCAGCGC CAATTACAGC AAAGCCTGGA AGCTTGACGC GCACCTGTGC 360

AAGCACACGG GGGAGAGACC ATTTGTTTGT GACTATGAAG GGTGTGGCAA GGCCTTCATC 420

AGGGACTACC ATCTGAGCCG CCACATTCTG ACTCACACAG GAGAAAAGCC GTTTGTTTGT 480

GCAGCCAATG GCTGTGATCA AAAATTCAAC ACAAAATCAA ACTTGAAGAA ACATTTTGAA 540

CGCAAACATG AAAATCAACA AAAACAATAT ATATGCAGTT TTGAAGACTG TAAGAAGACC 600

TTTAAGAAAC ATCAGCAGAT GAAAATCCAT CAGTGCCAGA ATACCAATGA ACCTCTATTC 660

AAGTGTACCC AGGAAGGATG TGGGAAACAC TTTGCATCAC CCAGCAAGCT GAAACGACAT 720

GCCAAGGCCC ACGAGGGCTA TGTATGTCAA AAAGGATGTT CCTTTGTGGC AAAAACATGG 780

ACGGAACTTC TGAAACATGT GAGAGAAACC CATAAAGAGG AAATACTATG TGAAGTATGC 840

CGGAAAACAT TTAAACGCAA AGATTACCTT AAGCAACACA TGAAAACTCA TGCCCCAGAA 900

AGGGATGTAT GTCGCTGTCC AAGAGAAGGC TGTGGAAGAA CCTATACAAC TGTGTTTAAT 960

CTCCAAAGCC ATATCCTCTC CTTCCATGAG GAAAGCCGCC CTTTTGTGTG TGAACATGCT 1020

GGCTGTGGCA AAACATTTGC AATGAAACAA AGTCTCACTA GGCATGCTGT TGTACATGAT 1080

CCTGACAAGA AGAAAATGAA GCTCAAAGTC AAAAAATCTC GTGAAAAACG GGAGTTTGGC 1140

CTCTCATCTC AGTGGATATA TCCTCCCAAA AGGAAACAAG GGCAAGGCTT ATCTTTGTGT 1200

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CAAAACGGAG AGTCACCCAA CTGTGTGGAA GACAAGATGC TCTCGACAGT TGCAGTACTT   1260

ACCCTTGGC                                                          1269
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1399 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:20..1288

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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ATGCGCGATC TCCCGGAGC ATG CGC AGC AGC GGC GCC GAC GCG GGG CGG TGC      52
                     Met Arg Ser Ser Gly Ala Asp Ala Gly Arg Cys
                       1               5                  10

CTG GTG ACC GCG CGC GCT CCC GGA AGT GTG CCG GCG TCG CGC GAA GGT     100
Leu Val Thr Ala Arg Ala Pro Gly Ser Val Pro Ala Ser Arg Glu Gly
             15                  20                  25

TCA GCA GGG AGC CGT GGG CCG GGC GCG CGG TTC CCG GCA CGT GTC TCG     148
Ser Ala Gly Ser Arg Gly Pro Gly Ala Arg Phe Pro Ala Arg Val Ser
         30                  35                  40

GCA CGT GGC AGC GCG CCT GGC CCT GGG CTT GGA GGC GCC GGC GCC CTG     196
Ala Arg Gly Ser Ala Pro Gly Pro Gly Leu Gly Gly Ala Gly Ala Leu
     45                  50                  55

GAT CCG CCG GCC GTG GTC GCC GAG TCG GTG TCG TCC TTG ACC ATC GCC     244
Asp Pro Pro Ala Val Val Ala Glu Ser Val Ser Ser Leu Thr Ile Ala
 60                  65                  70                  75

GAC GCG TTC ATT GCA GCC GGC GAG AGC TCA GCT CCG ACC CCG CCG CGC     292
Asp Ala Phe Ile Ala Ala Gly Glu Ser Ser Ala Pro Thr Pro Pro Arg
                 80                  85                  90

CCC GCG CTT CCC AGG AGG TTC ATC TGC TCC TTC CCT GAC TGC AGC GCC     340
Pro Ala Leu Pro Arg Arg Phe Ile Cys Ser Phe Pro Asp Cys Ser Ala
             95                  100                 105

AAT TAC AGC AAA GCC TGG AAG CTT GAC GCG CAC CTG TGC AAG CAC ACG     388
Asn Tyr Ser Lys Ala Trp Lys Leu Asp Ala His Leu Cys Lys His Thr
        110                 115                 120

GGG GAG AGA CCA TTT GTT TGT GAC TAT GAA GGG TGT GGC AAG GCC TTC     436
Gly Glu Arg Pro Phe Val Cys Asp Tyr Glu Gly Cys Gly Lys Ala Phe
    125                 130                 135
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ATC AGG GAC TAC CAT CTG AGC CGC CAC ATT CTG ACT CAC ACA GGA GAA      484
Ile Arg Asp Tyr His Leu Ser Arg His Ile Leu Thr His Thr Gly Glu
140                 145                 150                 155

AAG CCG TTT GTT TGT GCA GCC AAT GGC TGT GAT CAA AAA TTC AAC ACA      532
Lys Pro Phe Val Cys Ala Ala Asn Gly Cys Asp Gln Lys Phe Asn Thr
            160                 165                 170

AAA TCA AAC TTG AAG AAA CAT TTT GAA CGC AAA CAT GAA AAT CAA CAA      580
Lys Ser Asn Leu Lys Lys His Phe Glu Arg Lys His Glu Asn Gln Gln
        175                 180                 185

AAA CAA TAT ATA TGC AGT TTT GAA GAC TGT AAG AAG ACC TTT AAG AAA      628
Lys Gln Tyr Ile Cys Ser Phe Glu Asp Cys Lys Lys Thr Phe Lys Lys
    190                 195                 200

CAT CAG CAG ATG AAA ATC CAT CAG TGC CAG AAT ACC AAT GAA CCT CTA      676
His Gln Gln Met Lys Ile His Gln Cys Gln Asn Thr Asn Glu Pro Leu
205                 210                 215

TTC AAG TGT ACC CAG GAA GGA TGT GGG AAA CAC TTT GCA TCA CCC AGC      724
Phe Lys Cys Thr Gln Glu Gly Cys Gly Lys His Phe Ala Ser Pro Ser
220                 225                 230                 235

AAG CTG AAA CGA CAT GCC AAG GCC CAC GAG GGC TAT GTA TGT CAA AAA      772
Lys Leu Lys Arg His Ala Lys Ala His Glu Gly Tyr Val Cys Gln Lys
            240                 245                 250

GGA TGT TCC TTT GTG GCA AAA ACA TGG ACG GAA CTT CTG AAA CAT GTG      820
Gly Cys Ser Phe Val Ala Lys Thr Trp Thr Glu Leu Leu Lys His Val
        255                 260                 265

AGA GAA ACC CAT AAA GAG GAA ATA CTA TGT GAA GTA TGC CGG AAA ACA      868
Arg Glu Thr His Lys Glu Glu Ile Leu Cys Glu Val Cys Arg Lys Thr
    270                 275                 280

TTT AAA CGC AAA GAT TAC CTT AAG CAA CAC ATG AAA ACT CAT GCC CCA      916
Phe Lys Arg Lys Asp Tyr Leu Lys Gln His Met Lys Thr His Ala Pro
285                 290                 295

GAA AGG GAT GTA TGT CGC TGT CCA AGA GAA GGC TGT GGA AGA ACC TAT      964
Glu Arg Asp Val Cys Arg Cys Pro Arg Glu Gly Cys Gly Arg Thr Tyr
300                 305                 310                 315

ACA ACT GTG TTT AAT CTC CAA AGC CAT ATC CTC TCC TTC CAT GAG GAA     1012
Thr Thr Val Phe Asn Leu Gln Ser His Ile Leu Ser Phe His Glu Glu
            320                 325                 330

AGC CGC CCT TTT GTG TGT GAA CAT GCT GGC TGT GGC AAA ACA TTT GCA     1060
Ser Arg Pro Phe Val Cys Glu His Ala Gly Cys Gly Lys Thr Phe Ala
        335                 340                 345

ATG AAA CAA AGT CTC ACT AGG CAT GCT GTT GTA CAT GAT CCT GAC AAG     1108
Met Lys Gln Ser Leu Thr Arg His Ala Val Val His Asp Pro Asp Lys
    350                 355                 360

AAG AAA ATG AAG CTC AAA GTC AAA AAA TCT CGT GAA AAA CGG GAG TTT     1156
Lys Lys Met Lys Leu Lys Val Lys Lys Ser Arg Glu Lys Arg Glu Phe
365                 370                 375
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GGC CTC TCA TCT CAG TGG ATA TAT CCT CCC AAA AGG AAA CAA GGG CAA 1204
Gly Leu Ser Ser Gln Trp Ile Tyr Pro Pro Lys Arg Lys Gln Gly Gln
380 385 390 395

GGC TTA TCT TTG TGT CAA AAC GGA GAG TCA CCC AAC TGT GTG GAA GAC 1252
Gly Leu Ser Leu Cys Gln Asn Gly Glu Ser Pro Asn Cys Val Glu Asp
400 405 410

AAG ATG CTC TCG ACA GTT GCA GTA CTT ACC CTT GGC TAAGAACTGC 1298
Lys Met Leu Ser Thr Val Ala Val Leu Thr Leu Gly
415 420

ACTGCTTTGT TTAAAGGACT GCAGACCAAG GAGTCGAGCT TTCTCTCAGA GCATGCTTTT 1358

CTTTATTAAA ATTACTGATG CAGAAAAAAA AAAAAAAAAA A 1399

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ATGGTCAAGG ACGACA 16

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AATGAATTCA TAAGGACGAC ACCGACT 27

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CCTCCAAGCC CAGGGCCA 18

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CAGAATCGAT AGTGAATTCG TG 22

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CACGAATTCA CTATCGATTC TGGAACCTTC AGACC 35

Claims

1. A human transcription factor IIIA gene coding for the amino acid sequence shown under SEQ ID No. 1.

2. The human transcription factor IIIA gene according to claim 1 containing the nucleotide sequence as shown under SEQ ID No. 2.

3. The human transcription factor IIIA gene according to claim 1 containing a nucleotide sequence complementary to the nucleotide sequence according to SEQ ID No. 2 or containing both the nucleotide sequence as shown under SEQ ID No. 2 and the nucleotide sequence complementary thereto.

4. The human transcription factor IIIA gene according to claim 1 comprising modifications introduced by deletion, insertion and/or substitution of at least one nucleotide base coding for a peptide still having a biological activity similar to that of hTFIIIA.

5. Expression vector containing a human transcription factor IIIA gene according to claim 1.

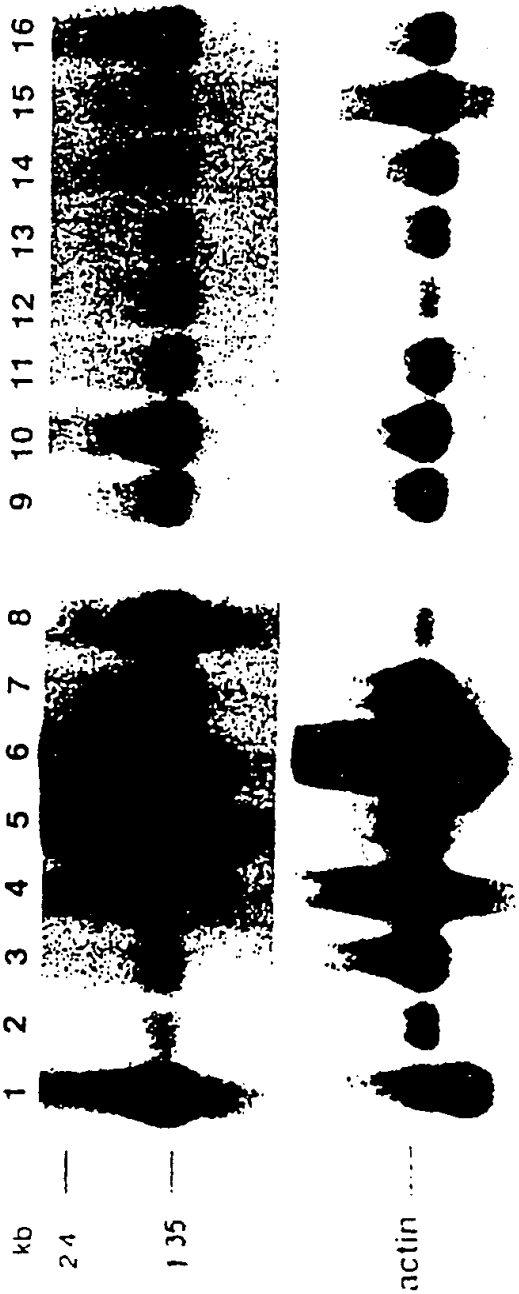
6. Process for producing a human transcription factor IIIA gene according to any of the claims 1 to 4 comprising selecting from among a human cDNA library a desired clone using an appropriate probe or antibody specific to the gene according to claim 1.

7. Process of preparing recombinant hTFIIIA comprising expressing the gene according to any of the claims 1 to 4 in an appropriate host and isolating and purifying the recombinant protein.

8. The use of a human transcription factor IIIA gene or a human transcription factor IIIA encoded by said gene for the preparation of a diagnostic or pharmaceutical useful for the diagnosis and treatment of diseases wherein a disorder in transcriptional control is involved.

9. Use according to claim 8 wherein the disease is related to cancer.

FIG. 1



European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 95 11 3908

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	J.BIOL.CHEM, vol. 269, no. 33, 19 August 1994 pages 20857-865, BETH MOOREFIELD ET AL. 'Purification and Characterization of Human Transcription Factor IIIA' * Material and Methods * * page 20864, last paragraph; table 1 *	1-9	C12N15/00 C07K14/46 A61K38/18
X	EMBL Database entry HS760 Accession number T15760; 05/08/1994 Stevens T.J. et al.: 'Gene- based STSs as the basis for a human gene map' * abstract *	4	
X	EMBL Database entry Hsgs00633 Accession number D19678; 23/06/1994 Okubo K. et al.: 'Gene expression of human promyelotic cell line HL60....' * abstract *	4	
A	EUR.J.BIOCHEM., vol. 196, 1990 pages 167-176, .ALDSCHMIDT ET AL. 'Physical and Immunological characterization of human transcription factor IIIA'		TECHNICAL FIELDS SEARCHED (Int.Cl.6) C07K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	20 December 1995	Deffner, C-A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
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